THE INTERACTION BETWEEN THE MALARIA PARASITE AND THE RED CELL MEMBRANE

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INTRODUCTION

This paper examines in brief the interaction between the malaria parasite and the red cell membrane, both during invasion and afterwards. The interaction during invasion involves the specific recognition process between the parasite and red cell surface, leading to endocytosis-like internalisation of the former. The post-invasion interaction is defined as that leading to modifications of the host red cell membrane in both structure and function, some of which may be of benefit to parasite survival while others may serve as signal for destruction by the host.

The molecular architecture of the red cell membrane is now known in considerable detail (see Schreier, 1985). The integral membrane proteins spanning both sides of the membrane proteins include band 3 and glycophorins. Band 3 is the major integral protein, and probably the major component of intramembrane particles. It is linked with spectrin, the major membrane skeletal protein, through another protein ankyrin (band 2.1). The glycophorins (A, B and C), of which 60% by weight is carbohydrate, are a group of proteins rich in sialic acid extending beyond the membrane surface. Glycophorins are linked with spectrin through the protein 4.1. The bulk of the membrane skeleton, underlying the inner membrane, is a network of spectrin and actin, also linked via protein 4.1. The interactions among these membrane proteins, and also between these proteins and the lipid components, are important in determining the various red cell membrane functions. These interactions, as well as interaction of individual components with the parasite surface must play an important role in parasite invasion.

In contrast to existing knowledge on the red cell membrane, very little is known about the merozoite membrane and other parasite components which take part in the invasion process, and less still is known about the plasma membrane of the intracellular parasite. The free merozoite has a plasma membrane with a glycoprotein surface coat, and two underlying pellicular membranes (Aikawa and Seed, 1980). The merozoite surface components which may play a part in the invasion and subsequent interaction with the red cell membrane include glycophorin-binding proteins (Perkins, 1984). A high molecular-weight antigen and its family of fragments located on the merozoite surface, found in many species of the malaria parasites (*P. yoelii*, MW 230,000; *P. falciparum*, MW 195,000; Holder and Freeman, 1984), are associated with protective immunity, but their role in the interaction between the merozoite and the red cell surface is unclear. A number of other protective antigens on the merozoite surface with poorly known function have also been identified (Anders, 1985; Newbold, 1984).

INTERACTION DURING INVASION

The invasion of the red cell by the merozoite follows a series of steps involving membrane

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processes (Miller, 1977; Aikawa and Seed, 1980; Breuer, 1985). Adherence to the red cell can occur on any part of the merozoite. When the apical end of the merozoite is orientated to red cell surface, there is widespread deformation of the red cell and a junction is formed at the contact point. The merozoite then enters into the endocytic invagination of the red cell, with the junction at the orifice moving parallel to the long axis of the merozoite. After the merozoite is completely engulfed, the red cell membrane fuses together sealing off the vacuolar membrane.

The adherence between the merozoite and the red cell results from the binding between the surface components of both partners. The host specificity of the process (Miller, 1977) indicates the presence of specific molecules involved. Specific molecules may also be involved in the subsequent processes of junction fromation and parasite entry. The junction is formed through clustering of intramembrane particles on the P(protoplasmic) face of the erythrocyte membrane while the vacuolar membrane was mainly devoid of the intramembrane particles (Aikawa *et al.*, 1981).

Characterization of surface molecules involved in the invasion process can be made by identifying genetically variant red cells which are less susceptible to invasion than normal, and studying the surface determinants involved. Inhibition of invasion following treatment of red cells with enzymes which degrade surface components selectively, or with antibodies specific to certain surface components, provides valuable clues. In both of these approaches uncertainty may be created by the presence of a secondary effect exerted by the variant or modified components which may not be directly involved in the invasion. Another approach is to study the inhibitory effect of added selected components isolated from the red cell membrane on the invasion process. In this case, it is important to avoid the possibility that the added components may exert an adverse effect on the development and viability of the merozoite.

The red cell surface components involved in invasion by *P. knowlesi*, and probably also *P. vivax*, have been shown to be associated with Duffy (Fy) blood group determinants (Miller *et al.*, 1975). Fy negative red cells are resistant to *P. knowlesi* invasion *in vitro*, apparently due to failure of junction formation (Miller *et al.*, 1979). Two antigens Fy^a and Fy^b are responsible for the phenotypic expression of the Fy blood groups, and anti-Fy^a and anti-Fy^b sera are able to block invasion of Fy (a^+b^-) and Fy (a^-b^+) red cells respectively. Invasion of Fy (a^+b^+) erythrocytes is blocked by treatment of the red cells with chymotrypsin. The Fy^a antigen has been characterized as a 35,000-43, 000 molecular-weight protein degradable by chymotrypsin (Hadley *et al.*, 1984). Athough the Fy antigens are associated with parasite invasion, their absence does not abolish all latent invasion susceptibility : treatment of Fy negative red cells with trypsin or neuraminidase renders the originally refractory cells susceptible to invasion. The Fy antigens therefore play an important role, though not an exclusive one, in determining the course of interaction between the red cell and the parasite leading to invasion.

Reduced invasion of *P. falciparum* in red cells devoid of, or defective in, certain blood group antigens (Miller *et al.*, 1977; Pasvol *et al.*, 1982) pointed to the possible role of glycophorins as a possible specific determinant of interaction leading to invasion. En (a^-) red cells lacking in glycophorin A, Tn red cells with underglycosylated glycophorin A, and $S^-s^-U^-$ red cells deficient in glycophorin B, all showed partial resistance to invasion. Invasion of normal red cells can also be inhibited after treatment with enzymes (trypsin or neuraminidase, but not chymotrypsin) which cleave glycophorin A. More direct evidence was obtained from the observation that glycophorin A or B or Fab fragments of

antibodies to glycophorin A added to the culture could effectively inhibit merozoite invasion (Perkins, 1981). The merozoite surface was shown to have glycophorin-binding proteins of molecular weights 155,000 and 130,000, the antibodies to which could effectively inhibit the invasion (Perkins, 1984). The gene for a glycophorin-binding protein has been cloned, and was shown to code for tandem repeats of 50 amino acids in a sequence with alternate hydrophobic and hydrophilic blocks (Ravetch *et al.*, 1985).

Although these results indicate a major role for glycophorins in specific interaction with the merozoite which triggers invasion, the mode of this interaction is still unclear. Since these proteins carry a major portion of surface sialic acid it might be expected that the binding domain should carry a high negative charge density. It should be noted that a number of macromolecules bearing negative charge could also compete with the merozoite-red cell interaction (Friedman, 1983). On the other hand, free sialic acid and glycoconjugates carrying terminal sialic acid residues fail to inhibit invasion (Deas and Lee, 1981; Breuer *et al.*, 1983). It was, furthermore, shown that up to 40% of sialic acid can be removed from the red cell surface by neuraminidase or trypsin before inhibition of invasion was observed (Olson, 1984), and that desialated glycophorin could inhibit invasion to a comparable extent as intact glycophorin (Breuer *et al.*, 1983). It is possible that more than one step is involved in the binding process, the exposed negatively charged domain providing initial but rather weak binding site, while the internal domain provides the more stable subsequent attachment site for the merozoite. Recent work has furthermore pointed to the role of band 3, the major transmembrane protein of the red cell, as a possible site for interaction with *P. falciparum* (Okoye and Bennett, 1985; Friedman *et al.*, 1985).

The physical state of spectrin, the major component of the membrane skeleton, is important for the subsequent interiorization of the merozoite, as shown by strong inhibition of the process following limited cross-linking of spectrin by chemical or immunochemical means (Dluzewski *et al.*, 1983a; Olson and Kilejian, 1982). Since spectrin is linked to the major transmembrane proteins, it may play a role in the redistribution of the intramembrane particles observed during the invasion. The membrane skeletal proteins could also play a role in modulating membrane deformability during invasion. It is of interest to note that ovalocytic erythrocytes from Melanesians are both resistant to invasion by *P. falciparum* and to temperature-induced deformation (Kidson *et al.*, 1981). However, hereditary spherocytes, also with reduced deformability, apparently have normal susceptibility to invasion (Koeweiden *et al.*, 1979).

Among the various other factors, intraerythrocytic, ATP was shown to be essential in promoting invasion (Olson and Kilejian, 1982; Dluzewski *et al.*, 1983b). The function of ATP is probably not related to the maintenance of cation gradients, but probably involves phosphorylation of membrane proteins, as suggested by the inability of non-hydrolysable ATP to replace ATP. Since shedding of the merozoite surface components occur during invasion, membrane-bound protease or glycosidase may be an important factor. The contents of merozoite rhoptries organelles near the apical end, discharged during invasion may also be critical for the process. Some rhoptry proteins have been shown to be protective antigens in *P. falciparum* (Holder *et al.*, 1985).

INTERACTION DURING INTRACELLULAR DEVELOPMENT

During intracellular development, many changes occur in the membrane of infected

red cells, including the structure and function of the protein components, the composition and arrangement of phospholipid components and the appearance of new parasite-derived antigens (Howard, 1982; Newbold, 1984; Sherman, 1984). Red cell spectrin has been shown to decrease with appearance of new lower molecular-weight proteins suggesting that spectrin degradation may occur during development of various species of malaria parasites (Weidekamm *et al.*, 1973; Konigk and Mirtsch, 1977; Yuthavong *et al.*, 1979). A cathepsin D-like protease has been purified from *P. lophurae*, and shown to produce similar degradative changes on the membrane proteins (Sherman and Tanigoshi, 1983). Degradative changes of membrane skeleton proteins may be linked with shape and deformability changes of infected cells, and may be relevant to the mechanism of merozoite release.

Red cell membrane proteins also undergo changes other than degradation during malarial infection. In *P. berghei*-infected cells, a protein of molecular weight 42,000 was shown to undergo phosphorylation (Chaimanee and Yuthavong, 1979; Wiser *et al.*, 1983). Various properties of this protein suggest that it is phosphorylated actin, and the level of phosphorylation is linked with osmotic fragility and filterability of the infected cells (Yuthavong, 1985).

Changes in antigenicity of the host red cell induced by the parasite has important implications in both immunopathology and in protective immunity. Some antigens appear early in the infection, e.g., the ring-infected erythrocyte surface antigen (RESA) in *P. falciparum* infection (Coppel *et al.*, 1984). The antigen appears to be identical to Pf155, a protein of molecular weight 155,000, the antibody to which could effectively prevent parasite invasion (Perlmann *et al.*, 1984). Other antigens appear in the later stages of infection. The variant antigen on *P. knowlesi*-infected red cells responsible for schizont-induced cell agglutination (SICA antigen) is one such example (Newbold, 1984). Another example is the antigen of molecular weight 285,000 responsible for cytoadherence of knobby red cells containing K^+ strains of *P. falciparum* (Leech *et al.*, 1984a). K^+ strains of *P. falciparum* has a histidine-rich protein (Kilejian, 1979) which binds to red cell membrane skeleton to produce cups as foci for knobs on the cell surface (Leech *et al.*, 1984b). It is perhaps not directly involved in cytoadherence but in presentation of the cytoadherence antigen. Cytoadherence is important for the sequestration of late-stage *P. falciparum*-infected red cells along the endothelium of small blood vessels, and may be a contributing factor to cerebral malaria. Furthermore, monocytes, platelets and a melanoma cell line which also bind infected red cells appear to use similar receptors for the binding (Barnwell *et al.*, 1985). Thrombospondin, a soluble adhesive glycoprotein present in blood, may also mediate cytoadherence (Roberts *et al.*, 1985).

Changes in the function of malaria-infected red cell membrane include enhancement in transport of cations, anions and metabolites. Increase in uptake of Ca^{2+} in red cells infected with various species of malaria parasites (Bookchin *et al.*, 1981; Leida *et al.*, 1981; Tanabe *et al.*, 1982; Krungkrai and Yuthavong, 1983) may be due to increased membrane permeability compounded by reduced pumping activity. Increase in permeability of anions and small metabolites may be due to increase in number of transport channels generated by parasite protein (Kutner *et al.*, 1985). The transport channels exclude disaccharides or larger molecules, and are positively charged (Ginsburg *et al*,. 1985). The selective transport increase probably serves important functions in supplying the parasite with required nutrients and ions for its metabolic purposes.

SUMMARY

The malaria parasite intimately interacts with the host red cell membrane throughout the cycle of invasion and intracellular development. Direct interaction between the merozoite surface and the red cell membrane involves specific binding between the surface components of both cells, which leads to the subsequent endocytotic process still incompletely understood. Intracellular development of the parasite is accompanied by various changes in the structure and function of red cell membrane components. Some changes may benefit parasite survival while others trigger host immune response. An understanding of both the direct interaction between the surface components of the parasite and the red cell during invasion, and the subsequent changes in the red cell membrane following invasion, should lead to better ways of controlling malaria.

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